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USSR WORK ON THE PROBLEM OF FORMATION OF MICROBIOLOGICAL SPECIES

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Darwinism dealt the final blow to the antiscientific, religious idea dominating pre-Darwinist biology that the species in nature were each created separately, not connected with each other in their origin, and that they were everlasting and unchanging. This metaphysical idea was replaced by Darwin's theories.

Darwin, however, did not arrive at the truly scientific ideology of dialectical materialism. Michurin, led by dialectical materialism, demonstrated that the development of living nature does not take place in the manner described by Darwin in his Origin of Species, i.e., that transmutation of species does not require thousands of years.

Scientists and agronomists, in the past few years, have discovered grains of rye in ears of wheat, grains of wild oats in cultivated oat plants, grains of brome grass in ears of rye, and grains of soft wheat in ears of hard wheat. Then, new, analogous facts were established. Barley plants discovered in wheat crops had grown from seeds which, in their external appearance, had differed in no way from wheat seeds. In wheat plants grown from one seed, one stem bore an ear of wheat, the other an ear of rye. In another case, the second stem bore oats. Even more interesting was the following fact: a plant was obtained from a grain of wheat, the ears of which belonged to three different biological species and five different varieties of wheat. These facts are not singular cases, and above all, they are not accidental.

In practice, the idea of regeneration or transformation of wheat into rye has been expressed for a long time.

All these similar facts, observed in the past and recently cleared up, have now produced a new explanation important to science and to practice, which leads to the fact that the origin of various contaminations and some impurities of crops [weeds] -- except for those frequently introduced by mechanical means -- may be the generation of one species of plants by another species.

What facts can be cited from the field of microbiology which gives us the right to state that transmutation of one species of microorganism into another species can be observed and even produced experimentally?

The greatest number of facts about modifiability was observed on bacteria which cause gastrointestinal diseases in men and animals (the greatest number of observations was carried out on these bacteria). Many reports have been published in literature on the so-called atypical strains, atypical cultures of the causative agents of typhoid fever, human cholera, and paratyphoid infections of humans and animals. In 1936, Professor P. S. Rozen wrote about this group of bacteria, after having devoted 10 years of study to the modifiability of the causative agents of intestinal infections: "It is often extremely difficult to tell where the atypical form of pathogenic microbes ends and where the saprophyte begins."

It has been shown many times that, for instance, typhus bacilli in the water supply may change their properties to such an extent that they will become indistinguishable from B.coli or from alkalizing bacteria: some strains even assume such properties (in particular, the capacity to reduce the viscosity of gelatine) which carry them outside the limits of the intestinal typhoid group. In by far not all such cases could the properties of the original typhoid fever bacilli be restored.

The reason for outbreaks of typhoid is most frequently the pollution of the sources of drinking water. Nevertheless, there are no more than a score of reports in world literature on cases where typical typhoid bacilli were isolated from the source of water supply. In the great majority of the outbreaks, even in those where the role of the water sources in originating the epidemic was quite certain, attempts to discover the typhoid bacteria failed.

It has been shown that typical typhoid bacteria were not found once even in such mass outbreaks as the typhoid fever epidemic at Hannover in 1926, although the water was examined literally by the bucketful. At that time, these facts gave some epidemologists, including prominent ones, reason to deny altogether the role of the source of water supply in the origin and spreading of typhoid fever.

Epidemology textbooks state that in samples of drinking water, typical typhoid fever microbes are found extremely seldom, because the water is examined too late, only after a serious suspicion has arisen that the cause of the outbreak should be sought in the source of water supply. At the time of the examination, flowing water (rivers, water mains) is no longer poluted but has become clear again and no longer contains the causative agents of the disease. In the great majority of cases, this explanation is, of course, well founded. Nevertheless, it must be pointed out that even in sources of water supply with stationary water, such as wells, typhoid bacteria have also been observed, on the whole, only rarely. This has been explained by the fact that the typhoid bacteria can live in drinking water for only a few days.

Among the many studies and experiments of water-borne typhoid infections, the following experiment by I. N. Blokhina (1), an associate of Professor F. T. Grinbaum, is of particular interest for the problems of species formation of microorganisms.

I. N. Blokhina infected sterilized drinking water with typhoid bacilli. After a few days, no typhoid bacilli could be detected in the water. Blokhina continued the seeding process. Bacteria which differed from the typhoid bacilli in many of their properties were soon isolated from the water. The new bacteria were not agglutinated by typhoid serum, and they were not virulent to mice. These

bacteria stayed alive for months, both in sterilized and nonsterilized water; they did not decompose the carbohydrates which are generally decomposed by typhoid bacilli, and furthermore they multiplied fastest at a temperature of 30° C.

By means of repeated reseedings in broth with horse serum, Blokhina again transformed the culture of this bacterium into the typhoid bacillus. She repeated the experiment many times with different strains of typhoid bacilli and always got the same results.

While carrying out these experiments, Blokhina investigated, in addition, the water of a well which had for a while been the source of typhoid infection. At a temperature of 37° C, typhoid bacilli were isolated from this water. In subsequent seedings at lower temperature (30° C), exactly the same cultures were isolated as those which had been obtained in the special experiment. The culture obtained was not agglutinated by typhoid serum but was agglutinated at high titers by serum which was specific for the bacterium that had been obtained experimentally. Although there are some reservations in regard to the method and conduct of the experiment, the results are nevertheless of undoubted interest to science.

Cultures which differ sharply from the typical causative agent of typhoid have been isolated a number of times by many Soviet and foreign microbiologists; frequently, moreover, not only from water, but also from the intestines and even from the blood of typhoid patients. The capacity of these cultures to be agglutinated by specific serum and to be dissolved by typhoid bacteriophage could be restored only infrequently.

The modifiability of species of the causative agent of human plague has been studied even more thoroughly and from more angles. We should like to cite the reports of G. N. Lenskaya, A. A. Bessonova (2), V. M. Tumanskiy, Ye. M. Korobkova, and M. P. Pokrovskaya who obtained bacteria from the causative agents of human plague which had all the properties of pseudotuberculosis of rodents. Plague microbes and pseudotuberculosis microbes are independent species. These microbes infect different animals. Their morphological, culture, and enzymatic properties are not the same, and if the two interact, interspecies competition takes place. As early as 1929, Bessonova, Lenskaya, Molodtsova, and Mosolova discovered five strains in old, standard cultures of plague bacteria, which behaved on all differential-diagnostic media like cultures of pseudotuberculosis of rodents. During the following 7 years, these cultures were examined thoroughly. The authors did not publish their findings until 1936, when they reported that they had succeeded in establishing the transformation of plague bacteria into pseudotuberculosis bacteria.

In the course of over 20 years, seven strains of pseudotuberculosis of rodents were thoroughly examined by various laboratories of the institute where the work was done. These strains were also obtained from cultures of plague bacteria. As far as enzymatic reactions are concerned, they differ in no way from pseudotuberculosis bacteria. When animals are infected with them, pathologicohistological changes take place, which are typical for pseudotuberculosis of rodents. The new cultures have the antigen specificity of the pseudotuberculosis bacteria, while morphologically, they are characterized by the presence of flagellae, which is unusual for plague bacteria. The first report of these experiments was then thoroughly checked at the institute and the results were reproduced several times in repeated experiments.

These and all the facts stated below on the modifiability of species in bacteria have now resulted in a new line of thinking, a new, scientifically correct approach to the problem.

There is a vast amount of publications on the modifiability of species, as far as the causative agent of human dysentery is concerned; the generally known causative agents of this disease are the Shiga-Kruse species, first discovered in Japan; bacteria of the Hiss-Flexner group, isolated in the Philippines; the Schmitz-Stutzer group, described in Russia and Germany; and the Kruse-Sonne type, discovered in the Scandinavian countries. Still other new types have been described in the past few years: Newcastle, Denton, and Novgorod; to the causative agents of dysentery also belong other species of bacteria: Morgan's bacillus and B.paracoli.

It is not an exaggeration to state that in no other group of pathogenic bacteria are there as many difficulties encountered in the determination of types and species, and that in no other group is there such a large number of variants and atypical strains as among causative agents of dysentery. It is often even hard to check whether some culture isolated from a patient has been identified correctly, because one variant will approximate the properties of another or develop new variants, even during a short period of storage.

The modifiability of dysentery bacteria was conclusively demonstrated in the experiments of Professor G. P. Kalina. His method, on the whole, consisted of exposing Flexner bacteria to the action of bacteriophage and subsequent cultivation and selection on special media. As a result, Kalina (4) obtained cultures of bacteria which were identical to paratyphus A. On having been stored for 9 months, they preserved the new properties. Kalina himself classified the new species which he had obtained as a stable variant of dysentery bacteria, merely because he had not answered the question as to whether this new microbe could cause paratyphus. However, undoubtedly, any worker in a diagnostical laboratory who finds the microbe described by Kalina in an external medium such as drinking water or in a patient will without further thought (and with complete justification) classify it as a member of the paratyphus bacteria group and not of the dysentery group.

There is also a great deal of published material on the high degree of modifiability and the mutual species transformations in the group of streptococci, staphylococci, and pneumococci.

Many researchers have described the mutual transition of pneumococci, Streptococcus haemolyticus, and Streptococcus viridans, both in experiments in artificial media and experiments on animals. This was noted, for instance, on cultivating pneumococci in symbiosis with hay bacilli, passing pneumococci through the organism of white mice, drying in organs of infected mice, etc. The mutual transition of Streptococcus haemolyticus into Streptococcus viridans is observed in scarlet fever patients; in the organs of infected mice treated with penicillin; in cultures grown on the saliva of healthy humans, after protracted growing of cultures in the serum of people who had recovered from scarlet fever; and on introduction of streptococci into immunized animals.

The so-called pseudodiphtheria bacteria have been described frequently and for a considerable time. They are frequently found in the mucous membranes of the nasopharynx, in the upper respiratory tract and the eyes of healthy children, in purulent processes in the pleural cavity, in cases of inflammation of the middle ear, in tubercular sputum, and on various wound surfaces. Such great microbiologists as Roux, Versen, Zlatogorov, and Behring considered these bacteria identical with the causative agent of human diphtheria, which causative agent had only temporarily lost its virulence. On the other hand, equally distinguished scientists such as Loeffler and L. V. Gromashevskiy, while admitting that diphtheria bacilli may lose their virulence, consider the pseudodiphtheria bacillus as a completely independent species which will not turn into the causative agent of diphtheria under any conditions. But here is what the clinicists have to say:

there had been no cases recorded for 40 years. After the most careful investigation, this experienced epidemiologist was forced to exclude the possibility of the disease having been brought in from the outside by animals or with the fodder.

A significant explanation of the fate of the anthrax bacilli in the ground was given by Professor K. A. Mirotvorskiy in his experiments (12). He established that in the soil, the anthrax bacilli not only are preserved for a long time and multiply, but, in addition, exhibit stable deviations from the original culture. In cases of the less pronounced deviations, the culture can be restored to the original state. He discovered nonvirulent cultures with profound and permanent deviations which could not be made to revert to a state corresponding to that of the original strain. Mirotvorskiy also established similar modifiability phenomena by seeding filtrates of samples of various soils with spores. In his conclusions, he indicated that virulent anthrax cultures which have lost their virulence may recover it in the soil.

Consequently, direct experiments have indicated that the causative agent of anthrax can be transformed into another species, a saprophytic microbe, both in artificial cultures and in an external medium. Furthermore, the results presented above do not refute but strengthen the theory of the possibility of transformation of saprophytic soil microbes into the causative agent of anthrax.

As early as 1898, Professor N. N. Khudyakov was convinced that aerobic forms of obligate anaerobes exist in nature. The views expressed by Khudyakov and his experiments induced the author of this article to conduct experiments on the cultivation of pathogenic anaerobes under aerobic conditions. These experiments were carried out during 1925 - 1930 (7). We started from the following considerations. Pathogenic anaerobes (e.g., tetanus bacilli, causative agents of gas gangrene) are permanent inhabitants of cultivated soil, and from there they get into the intestinal tract of animals. They can be found in nearly all samples of surface soil.

According to general opinion, the life activity of anaerobic microorganisms in the soil is aided by aerobic microbes which absorb the free oxygen of the soil and thus provide oxygen-free conditions. Accepting this explanation means assuming that the aerobes assimilate a tremendous quantity of atmospheric oxygen in the uppermost layers of the soil, where it is admitted constantly from the atmosphere.

In the upper layers of cultivated soil and even in the deeper ones, there is not and there cannot be any such complete lack of oxygen as we can create in the laboratory, where the pressure in apparatus for growing anaerobes is reduced to a few millimeters of mercury. It would be more correct to assume that aerobic microorganisms neutralize or use up metabolism products harmful to anaerobes in the presence of oxygen or that they produce such nourishment for anaerobes as will not form harmful products on assimilation by them.

This explanation can be fully verified experimentally. We attempted to produce a nutrient substrate in which anaerobic microorganisms could live and grow while atmospheric oxygen had free access.

From the start, we refrained from using laboratory cultures of pathogenic anaerobes, since through many generations, they had been maintained and had multiplied under oxygen-free conditions. The initial tetanus culture was obtained from a sample of vegetable garden earth. The spores of the causative agent of tetanus can withstand prolonged boiling (for several hours). Into a half-liter vessel containing a special medium (meat broth fermented with yeast, 0.5% NaCl, 0.5% $Na_2S_2O_3$, 0.05% iron pyrophosphate, pH = 8.2), we introduced 50 g of well-fertilized earth. We boiled the infusion for 40-50 min and then placed it into a thermostat for 5-7 days at 40-42° C.

Part of the culture was filtered through a Seitz filter, and the filtrate was injected into white mice or guinea pigs to determine the presence of tetanus toxin. If the animals died with characteristic tetanus symptoms, a reseeding was carried out from the extract into the original medium (5-10 cc), but without earth. Prior to the seeding, the medium was heated to 100° C to kill any nonsporiferous microbes which might have been present. Within 5-7 days, while the drum-type bacillus forms typical for tetanus were present, the culture was seeded under normal aerobic conditions on a Petri dish with a solid medium of the same composition, and sterile soil extract was added.

Using the above method, we succeeded in obtaining a pure culture of tetanus bacilli from three out of six samples of soil and from two out of four samples of horse faeces. In the liquid medium, the cultures of bacilli did not differ in their morphology from the typical anerobic cultures; on aging, elongated units appeared in the culture, showing grains interwoven with threads. On solid agar-agar, under customary aerobic conditions, young colonies more frequently had the form of cloud-like turbidities with round or irregular edges. After several days of growth, the colonies became grey in the center, with light edges; old colonies consisted of belts of various colors (brown, grey), with small, very thin offshoots.

On seeding with a needle, the typical growth in depth was observed, and also growth on the surface. In liquid media, aerobic cultures produced specific tetanus toxin. Cultures grown on solid agar-agar subsequently grew on all customary solid and liquid media with free access of air. They showed a reaction with catalase and did not decompose carbohydrates. When aerobic cultures were stored under paraffin, they retained their toxigenic properties for more than a year. Old aerobic agar-agar cultures which had not been reseeded for a long time, and also cultures which had been reseeded many times, gradually lost their toxigenic properties on solid media (up to complete loss). According to morphological and culture properties, these cultures approached more and more closely the spore-producing soil microbes of the species of hay bacillus.

A second series of experiments was carried out with the chief causative agent of gas gangrene, B.perfringens.

This microbe is found in nearly 100% of all samples of cultivated soil. As far as its fermentation characteristic is concerned, it is frequently classified as a butyric-acid microbe, since among the final products of the fermentation of carbohydrates, it forms a large quantity of butyric acid (from which stems one of the synonyms designating this microbe: Saccharobutyricus immobilis). There is even more reason for placing it in the group of the so-called pseudolactic-acid bacteria which, in the fermentation of carbohydrates (lactoses), produce a large quantity of butyric and acetic acid, in addition to lactic acid. According to these considerations, we also decided to use the method employed for lactic-acid bacteria to cultivate B.perfringens.

After trying a number of different nutrient media, we arrived at the following composition: yeast decoction (pH = 8.0 to 8.2)-- two parts; sterile whey or milk-- one part. Chalk was added to neutralize this.

We started with laboratory cultures but quickly went over to obtaining strains from natural substrates, i.e., soil and horse faeces.

An emulsion of soil or faeces in distilled water was heated for 20-30 min at 80° C to free it from nonsporiferous microbes. After the larger particles had been allowed to settle, a milk-yeast medium was seeded with a few drops of emulsion from the upper layer. The seeded flasks were put for 6-8 hr into a thermostat having a temperature of 40-42° C and kept under customary aerobic conditions. Subsequent reseedings were carried out after 3-4 days. After 3-4

reseedings, cultures were seeded in Petri dishes, also under ordinary aerobic conditions. The formula of the agar-agar was yeast extract with addition of 1/3 (by volume) of whey or animal blood serum; lactose (or better, maltose), 2%; and agar-agar, 2%.

We used the same method to obtain cultures of B.perfringens from clinical material.

In our further work on the transformation of pathogenic anaerobes into aerobic microbes, we became convinced that sporous forms freshly isolated from natural substrates are the most suitable for this purpose.

All aerobic B.perfringens cultures which we obtained grew quickly and profusely both in liquid and on solid media under free access of atmospheric oxygen. On a solid medium, the colonies were moist, usually round, with even edges and a slightly raised center; old colonies had a convex, opaque center, and a jagged, fine-grained edge. On agar-agar with blood, the colonies were surrounded by a greenish, semitransparent belt. Aerobic strains decomposed glucose, maltose, saccharose, and lactose but did not decompose mannitol. Fermentation of milk, as well as of other carbohydrates, on seedings from old cultures (especially those from solid media) was sharply retarded, and occasionally did not take place at all.

The microscopic morphology was as follows. In tissues and exudates taken from laboratory animals that had died from the infection with aerobic cultures, typical, even bacilli surrounded by capsules are noted. In cultures from organs in liquid and especially on solid media, coccic forms are found in addition to typical bacilli. Spore formation in aerobic cultures sets in considerably earlier and proceeds further than in anerobic cultures, and the spores may be encountered even in the organs and exudates of infected animals. After many reseedings on solid media, the aerobic cultures acquire mobility.

Freshly obtained cultures were virulent, while the old ones, especially if they had acquired coccic forms, were low in virulence or altogether nonvirulent. Animals (guinea pigs) infected with cultures which had not been reseeded under aerobic conditions for a long time died, despite the fact that they did not exhibit the characteristic local afflictions.

In the years during which these experiments were conducted, we had only one aim: to show that the capacity of anaerobic microorganisms to live only in the absence of atmospheric oxygen can be changed by the conditions of life. We succeeded in creating such conditions for anaerobes under which atmospheric oxygen ceased to be a poison for them and instead became a necessity of life. We used every possible control to prove that there had been no accidental contamination. We wrote in our conclusions: "We believe that the possibility of obtaining aerobic growth of pathogenic anaerobes, depending on the chemical composition of the nutriert substrate, is an affirmative answer to the question as to whether aerobic forms of these microorganisms exist in nature."

It is quite clear that the aerobic cultures which we obtained and which possess such profoundly changed types of respiration and other properties must not be considered merely saprophytic forms, varieties, or stocks of the identical species. They are entirely new species of microorganisms, different from the original microbes.

Examples of the transformation of anerobic microorganisms into aerobic ones can be cited also for saprophytes. Already in 1909, Bredeman grew amylobacter under conditions involving free access of atmospheric air. These observations were confirmed in 1927 by Cunningham and Jenkins. In 1940, Rotmistrov turned

cellfalcicula into aerobes by gradually increasing the amount of atmospheric oxygen admitted. Aerobic cultures of these microorganisms differed sharply from the original cultures in their biological and morphological properties, in connection with the changed type of metabolism.

Quite recently, there appeared a publication by L. D. Shturm on the obligate anaerobe V.desulfuricus, a microorganism which reduces sulfates. Shturm isolated a culture of this microbe from water in the stratum of tertiary deposits of the oil field of Changyr-Tash at a depth of about 900 m. On seeding with a large quantity of this culture, she succeeded in growing these bacteria on the surface of meat-peptone agar-agar. The aerobic cultures of the bacteria which reduce sulfates retained the capacity to develop under conditions involving free access of atmospheric air.

The work of M. R. Preobrazhenskaya on the study of the biology of iron bacteria belongs in the same category. She demonstrated that iron bacteria, classified by systematologists as aerobic microorganisms, will actually multiply even in the depth of springs, forming colorless flake-like colonies.

Our experiments and these short references from the literature permit us to assume that both in anaerobes and aerobes, the type of respiration can be profoundly changed, while in connection with this, the other properties characteristic for the species of the original microbe will also change.

We must not overlook the work of Professor V. I. Kedrovskiy on the modifiability of the causative agent of tuberculosis. On the basis of his experiments, carried out over many years, he came to the conclusion that the causative agent of tuberculosis exists in the environment in the form of a saprophytic, ordinary ray fungus. On the basis of his observations and the results of the work of other investigators (Ferran, Kumbari, A. I. Togunova, and others), Kedrovskiy gives the following explanation: "The universal distribution of tuberculosis cannot be explained from the viewpoint of recognition of only one classical form of the causative factor of the tuberculosis agent which we all know, i.e., the acid-resistant bacillus which lives in a strictly parasitic fashion at a high temperature and on nutrient media high in fat and nitrogen content. In addition to this form, one must acknowledge the existence of a second form, i.e., a saprophyte which may live and stay alive under conditions approximating those of inanimate nature."

Kedrovskiy arrived at this conclusion after more than 20 years' study of the biology of the causative agents of tuberculosis and leprosy. "I did not want to report my observations in print," wrote Kedrovskiy in 1935, "because they had been carried out in the period of the complete domination of the theory of monomorphism of microbes" (3).

Subsequent work by Togunova, Ramner, Trius, Berger, Mazur, Veysfeller [Weissfeller?] Kleptsova, Zhmolina, and many others has shown that in addition to typical bacilli, forms of tuberculosis bacilli that are not resistant to acid as well as acid-resistant pigmented bacteria are found not only in laboratory cultures but also in the blood, sputum, and urine of tuberculosis patients. Of these bacteria, some retain residual virulence, while others are complete saprophytes. The majority of earlier and present-day microbiologists, however, think that this is merely a matter of splitting off of variants or stocks.

The list of analogous material obtained by medical and veterinary microbiologists could be considerably expanded. In general, there are not many infectious diseases, in connection with which, in addition to the generally recognized causative agents, no so-called false strains, pseudo strains, or para-strains have been described, e.g., paracoli, paratyphus bacteria, pseudodiphtheria, pseudotuberculosis, false anthrax bacilli, false glanders bacilli, paradysentery, and various nonvirulent variants.

Many microbiologists have thought or think quite correctly that the overwhelming portion of these para and false strains or variants are connected by their origin with the common disease-producing agents. This idea has been confirmed many times by irrefutable direct experiments and observations, which left no doubt that pathogenic bacteria which have gotten into the external medium (soil or water) from the infected organism will be changed in the direction of acquiring saprophytic properties until they have been completely transformed into saprophytic microbes. The conversion of the pathogenic microbe into a saprophyte in the external medium evidently is facilitated by the fact that the process is essentially, so to speak, a return of the pathogenic species to the previous, natural saprophytic form of life which presumably already existed.

Nevertheless, the majority of microbiologists absolutely denies the possibility of the reverse process, i.e., the transformation of saprophytic microbes into existing and known pathogenic species under present-day conditions. Even putting the question of such a possibility allegedly indicates some kind of disorientation among practical workers, which undermines the basis of the prophylactic measures in medicine, veterinary medicine, and phytopathology. Any such transformation is admitted to have taken place only as a consequence of many centuries of evolution, as the result of a prolonged, gradual adaptation of saprophytes to parasitic life in the organism of higher animals and plants.

There are only a few microbiologists and epidemiologists who think that transformation of saprophytic species of microbes into pathogenic species under present-day conditions is possible and who have conducted experiments in that direction. Thus, Professor Zlatogorov, in 1908, and Professor Gorovits-Vlasova, in 1911, on the basis of investigations of the modifiability of the cholera vibrio,, expressed the idea that the harmless saprophytic vibrios in the human intestine may, under especially favorable conditions, turn into true cholera bacilli. Professor Zlatogorov also admitted the possibility of the endogenous development of human dysentery, i.e., as a result of the development of specific pathogenic properties in harmless intestinal bacteria.

We have already mentioned similar ideas relating to the causative agents of diphtheria and anthrax.

Under all conditions, transference of such scientific assumptions to practice requires great caution. The decisive role of the infected organism (of humans, animals, or plants) must not be underestimated to any extent in the development and spread of infectious diseases. A sick organism, or one which has recently been sick, remains the chief source of the spread of infectious disease. Practice has shown that in all cases, antiepidemic measures which are carried out properly and at the right time will stop outbreaks of infectious diseases. This approach determines and will determine the basis of all practical measures for prevention and liquidation of infectious diseases.

While admitting the possibility of the generation of pathogenic species by saprophytic microorganisms, the role of the preventive and sanitary measures tried and checked in practice in the fight against infectious diseases of humans, animals, and plants must not be minimized. The discovery of the conditions under which the transformation of a saprophytic microbe into a pathogenic species and vice versa is possible only helps to equip practice with supplementary means that permit prevention of the generation of infectious diseases. Thus, on discovering the conditions which favor the generation of brome grass from rye, T.D. Lysenko suggested an effective method for fighting the contamination of rye with brome grass.

It is certain that saprophytic microorganisms have profound species differences distinguishing them from pathogenic microbes and that the transformation of saprophytes into pathogenic species and altogether different species certainly

does not take place easily and frequently in nature, occurring only in cases when a whole complex of particularly favorable conditions accumulates. The presence of remnants of tissue, organs, or secretions of animal or plant organisms in the environment should be considered an essential element among the many factors and conditions necessary for such a change.

It is further possible that suitable conditions for the generation of some pathogenic microbes no longer exist. This fact, apparently, also explains why some infectious diseases have disappeared or are on their way out. The form of dysentery caused by the Shiga bacteria may serve as one of the most recent examples. Not long ago, still within our own memory, Shiga bacteria played a chief role in the etiology of acute dysentery, while now, this microbe can be found only in collections of standard cultures.

It is difficult to imagine the appearance and development of sets of enzymes in saprophytes which endow the saprophytes with the capacity to live and multiply in the organism of a plant or animal, unless the saprophytic microorganisms are in contact with a microorganism that has been weakened for some reason.

This can be explained and illustrated by examples. A group of so-called conditionally pathogenic microorganisms has been known for a long time. This term denotes microbes which are capable of exhibiting their pathogenic activity only in case of general weakening of the organism of the animal or plant or in the case of weakening (injury) of individual organs or tissues. Even B.coli, that typical intestinal saprophyte, and in the majority of cases, even a useful bacillus, can give rise to a number of inflammatory processes which may even include a fatal general blood infection when the organism is weakened.

There are well-known examples of the pathogenic action of staphylococci and streptococci on animals and of soil saprophytes on plants, although these microbes are usually harmless to a healthy organism.

In concluding this section, we will briefly touch on the question of the nature of the so-called live attenuated vaccines, on which work has been considerably intensified in medicine and veterinary medicine at present.

Both from the scientific and especially from the practical viewpoint, it is extremely important to determine just what a vaccine strain used for inoculations of humans and animals represents from the standpoint of its species characteristics: is it a new species of microorganism, merely a variant of the causative agent of the disease, or, finally, a live vaccine, i.e., an ordinary culture of the causative agent with its virulence somewhat attenuated? The development of the correct method of inoculation and of instructions for the production of a vaccine depend to a great extent on the classification given to a certain live vaccine.

This question, which is of great theoretical interest and practical importance, requires special elucidation. Here, we shall limit ourselves to the observation that the nature of the present-day, so-called live attenuated vaccines is certainly inhomogenous.

In my opinion, anti-anthrax vaccine, let us say, must not be considered a stock or a variant of the causative agent of anthrax. This vaccine has lost the species characteristics inherent in any stock or variant of the anthrax microbe: it does not produce anthrax with its characteristic clinical picture and epidemiological peculiarities in animals that have been inoculated with it.

The vaccine strain used in practice for inoculations against anthrax is a new species of microorganism, close to the anthrax bacillus, which does not exist in nature and which has been created by man. It is not a stock of the anthrax bacillus.

On the other hand, the US brucellosis strain (the so-called Strain No 19) must not be considered a new species in relation to the causative agent of brucellosis of cattle. Strain No 19, introduced into pregnant cows, can give rise to the typical brucellosis miscarriage and is entirely analogous to the natural disease, as far as the clinical picture and danger of spreading of the infection by animals having miscarriages are concerned.

From the biological standpoint, these two live vaccine strains [anthrax and brucellosis] are essentially different.

Many examples of species modifiability can be cited from the fields of soil microbiology and technical microbiology.

The greatest amount of experimental work has been carried out on the sporiferous soil microbes B.mycoides and B.mesentericus.

Many domestic and foreign investigators have obtained stock of B.mycoides, and B.mesentericus which did not differ from B.effusus, B.olfactorius, B.cereus, and B.brevis; variants of B.megatherium have been described which are analogous to B.tumefaciens, B.ruginatus, and B.pansini. There are indications of the transition of Streptothrix into Streptomyces and Streptoforus; variants of Pseudomonas aurantica have been isolated, which do not differ from natural strains of the species Pseudom. fluorescens. Even greater modifiability has been established in the class of actinomycetes. There are fungi in this class, which are in many respects identical to molds (actinomycetes), and on the other hand, there are those which resemble yeast-like fungi (mycobacteria and mycococci).

A great deal of work has been devoted to the modifiability of azobacter. Thus, it has been found that when it is grown on media that are rich in nitrogen compounds, stocks can be obtained which do not have the capacity of fixing nitrogen and which will not grow on media lacking nitrogen. In addition to that, it has been shown to be possible to force some bacteria which do not assimilate nitrogen from inorganic compounds to assimilate nitrogen in that form, if they are grown on media that contain inorganic nitrogen salts. This has been shown by Professor Krasil'nikov in experiments with various species of saprophytic microorganisms belonging to the genera Pseudomonas, Bacterium, and Bacillus (5). In two Pseudomonas cultures, Krasil'nikov produced the capacity to form bulbs on clover roots, and on another culture, he produced the capacity to form bulbs on lucerne roots. These cultures firmly retained the properties of bulb forming bacteria in succeeding generations, not only when reseeded onto plant roots, but even when grown on artificial laboratory media.

While the work on the study of the modifiability of the sporiferous soil microbes B.mycoides and B.mesentericus did not involve practical questions and has no generally recognizable importance, the work of which we have just spoken is of direct practical interest.

The following shows how profoundly the type of metabolism of microbes can be changed. All known microorganisms have for a long time been divided into two large groups: prototrophs, which use inorganic matter for nourishment and energy processes; and heterotrophs, which require organic compounds.

The principle of the strict division into two such groups has been in doubt for a long time due to a large number of facts which testify to the radical modification of nutrition and the whole type of metabolism, even in the most typical representatives of these groups. As early as 1914, A. F. Lebedyev discovered that the fungus Aspergillus niger is capable of assimilating carbon dioxide, despite the generally prevailing opinion that heterotrophic microorganisms assimilate carbon only from organic compounds. The capacity of synthesizing many

organic compounds has been established also in some pathogenic bacteria (e.g., tuberculosis, diphtheria), despite the fact that pathogenic microbes should apparently require for their development only complex organic compounds entering into the composition of the body of animals and plants.

We are reminded of the fact that nitrogen-fixing bacteria in protein media lose their capacity of assimilating molecular nitrogen. It is known that photosynthesizing microbes will turn to heterotrophic nourishment in the absence of light, sulfur bacteria which oxidize sulfur compounds can change their type of metabolism to the same degree, and anaerobic microbes for which atmospheric oxygen is a poison can be modified into aerobes so that oxygen becomes essential to them.

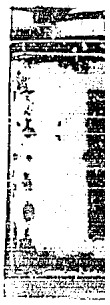
All these facts of the species modifiability of microorganisms are no less striking than the change of the nature of pathogenic microorganisms of which we have spoken earlier.

The factual material presented above leaves no doubt that Lysenko's rules of species formation in higher plants have also been fully verified on microbiological objects. It must be realized that the view prevalent among microbiologists to the effect that existing species of microorganisms were created only in the remote past and that none are being created at the present time is unscientific. Species formation of microorganisms is taking place also at the present time. Lysenko wrote, referring to the facts about the transformation of cereal plants species: "Many, if not all existing species can be generated again at the present time, and under proper conditions can be repeatedly generated by other species" (9). The material presented by us shows that these facts cannot only be observed but also be reproduced experimentally, as far as microbiological objects are concerned.

In his work New Findings in the Science of Biological Species, T. D. Lysenko exposed the conditions and explained the nature of the changes in species characteristics of organisms. He stated: "Changes in the conditions of the external medium responsible for the species characteristics of given organisms will sooner or later compel a change in the species characteristics -- one species generates another. Under the influence of conditions which have changed and have become unfavorable to the nature (heredity) of the organisms, rudiments of other species which are better adapted to the changed external conditions are generated in the body of the organisms of these species" (9).

In microbiology, up to now, no systematic, thorough work has been developed either on the explanation of those definite conditions under which one species of microorganism will generate another species or on the question of what particular species will generate certain other species. Most microbiologists, in their statements on the modifiability of microbes, limit themselves to the use of the vague term "variant," and try to concern themselves as little as possible with actual data and with the question of the possibility of transformation of one species of microbe into another. Other microbiologists, in their works, make no distinction between stages of development and species modification. They are inclined to regard rickettsiae of typhus fever and Proteus X as stages of the ontogenetic development of the same microorganism and not as different species, which is what they really are. And finally, there are some who deny species differences even between visible bacteria and ultraviruses and claim that it is possible in nature for one species of microbe to change, substantially, into any other species. In this, the teachings of Lysenko, which are of general importance to biology and state that "one plant species may generate various species which are close to it" (9), have been definitely disregarded.

One of the greatest achievements of Michurinist biology is the fact that it acknowledges the existence of species and also the interdependence of the links of nature. It has also introduced and substantiated the following new postulate:



"The qualitative difference between intraspecies interdependences and interspecies interdependences is one of the most important criterion for distinguishing species from varieties" (T. D. Lysenko). This concept of the problem of intraspecies and interspecies interdependences is extremely important for correct systematization and classification of species of animals, plants, and microorganisms. This has been of great help in agricultural practice in solving the most important problems connected with the cluster method of planting forests, kok-saghyz, and vegetable cultures, fighting contamination with weeds; and selecting components of grass mixtures.

Among plants and animals, great differences between species which are close to each other, such as the inability to crossbreed under natural conditions and antagonistic relations, are in general easily noticed by scientists and practical workers. It is even easier to distinguish species which are less close to each other.

As far as single-cell microorganisms are concerned, separating them into species is often difficult at the present stage of our knowledge, in view of the specificity of the objects of investigation [sic], while all concrete definitions of the concept of species are still less satisfactory.

It should be pointed out, however, that even among bacteria, i.e., in the group of microorganisms which is least differentiated by science, distinguishing of species which are not close to each other is no more difficult than distinguishing of higher plants. Any laboratory worker can easily tell anthrax bacilli from intestinal bacteria, or staphylococci from diphtheria bacilli when they are grown on media commonly used for them. As for species which are close to each other, the description of features and properties of individual species given by science should be re-examined in the light of Michurin's general biological theory of interspecies and intraspecies interdependence in the animal and plant world. In re-examining M. I. Shtutser and N. A. Krasil'nikov's (6) definitions of species on this basis, the concepts of bacterial species can be defined as a complex of ecological varieties or stocks which have in a natural medium a complex of similar and hereditarily strengthened features and properties (morphological, growth, physiological, and serological characteristics) and which exhibit no antagonism to each other in their interrelationship. Naturally, as knowledge develops, new qualitative species differences will become apparent, and the understanding of already known species features and properties will increase.

An important correction should now be introduced also into the understanding of the term "genus," as applied to microorganisms. On the present-day level of the theoretical development of the question of species formation, based on the fact that "an individual of a given species can be generated not only by the given species but also by individuals of another species, but obviously not any other species" (10), it must be admitted that the term "genus," by which similar species are brought together according to present-day classification, cannot always express the philogenetic connection representing the common root of the origin of species that are close to each other. Thus, genetically, the group of cocci bacteria is absolutely nonhomogenous. It is entirely possible, for instance, that some species of cocci are derived from mycococci and enter into the class of actinomyces (N. A. Krasil'nikov). The origin of the group of anaerobic cocci is not clear. Most likely, they do not all have the same origin. Further observations will undoubtedly permit the collection of data on the fact that also some other groups of microorganisms united into one genus by the present system will turn out to be unrelated from the standpoint of their origin.

On the other hand, species which according to the present-day system belong to different genera are related and have sprung from the same origin. Thus, as early as 1909, Bredemann, on the basis of his own careful investigation and

of all material available to him, arrived at the conclusion that the majority of the butyric acid bacteria described, of which some have been held to belong to different genera, really are all of them varieties of the same species of B.amylobacter.

V. L. Omelyanskiy, S. A. Korolyev and others have also spoken of the incomplete development of the systematics of lactic acid and butyric acid bacteria from this viewpoint.

Greater clarity can also be introduced into the concept of variety, as applied to the objects of microbiological study.

Under natural conditions, in nature, varieties are forms of existence of a given species under different ecological conditions that came into being as a result of an extensive diversity of ecological adaptations. These forms do not complete but contribute to the preservation and flourishing of the species. In the present-day system, such microbes as Azobacter chroococcus, Azobacter nigricans, halophilum, agile, and vinelandi are incorrectly classified as independent species. All these nitrogen-fixing bacteria are only ecological stocks of the same species, but they are by no means separate species. By the same token, there is no basis for classifying the bulb bacteria of clover, lucerne, lupine, etc., as separate species, only because they grow on the roots of different leguminous plants.

It is still more incorrect, I think, to classify as an azobacter stock, or even more so, as an azobacter strain microbes which have lost their capacity to assimilate atmospheric nitrogen and have hereditarily fixed this new property. Such cultures have, biologically speaking, lost the right to carry their previous generic name of azobacter.

The serological and immunogenic type specificity and the presence of phagotypes within one species are of no lesser scientific and practical interest. The intraspecies type specificity is best studied on bacteria pathogenic to humans. Probably one of the clearest examples of the multiplicity of serological types within one species is the species hemolytic streptococcus, which has about ten serological types distinguished on the basis of specific polysaccharides. One of them, type A, in turn is divided, according to the presence of specific proteins, into more than 40 serological types. The origin of this type specificity is still not clear.

From a theoretical and especially from a practical standpoint, it is most important to outline ways of directing the species formation of microorganisms. This will determine the trend of future work.

Much material has been gathered in microbiology on the precell filterable forms of microbes. This material leaves no doubt that the most promising way toward the experimental changing of the nature of microorganisms leads through the precell forms.

In the development of precell filterable forms, as a rule, cells are obtained which in some properties differ to a certain degree from the original microbe culture. This has been observed by every microbiologist who has ever worked with filterable forms of microbes. The first generations of microbe cells obtained through regeneration of filterable forms, the so-called secondary cultures, have the capacity to revert to the original type but on the other hand, are much more easily subjected to further change, as far as the species characteristics of the original microbe are concerned. Therefore, microbe cells from secondary cultures are particularly suitable for profound directed modification of the nature of microbes.

Microbiologists have gathered most of their experience in obtaining precell filterable forms of microbes by having a phage act on the original vegetative forms. The foundations for this work were laid by V. V. Suknev. In all the cases cited above, in which the agent of pseudotuberculosis of rodents was obtained from cultures of plague bacteria, or in which the dysentery microbe was turned into the paratyphus microbe, the action of a specific phage on the original culture was used.

The transformation of ordinary visible microbes into the precell forms is also accomplished by means of other actions exerting an unfavorable influence: repeated freezing and thawing, action of specific bacteriolytic sera, antibiotics, antiseptics, various salts, prolonged keeping of a culture without reseeding, etc.

The method of regenerating precell forms has also been developed to a higher and higher degree. It is extremely important to learn to conduct the regeneration of the precell forms and the raising of the first generations of secondary cultures under conditions which would guarantee the purposeful change in the nature of the microorganisms required by practice. Of course, it is impossible to indicate or foresee the conditions and the time of breeding, the number of changes of generations, the selection of the original culture, etc., for any particular microbe.

The conditions and factors of species modifiability are manifold. But despite their multiplicity, the basis of origination of a new species is the fact that the elements of the external medium (aeration, moisture, temperature, sources of nourishment and of energy processes, biocenosis, etc.) are not favorable to the nature of the old species and favor the origination and development of new species qualities against the background of the old species. No matter how great the effect of biocenosis may be on the change of the nature of microorganisms under natural conditions, species modifiability can and should be studied also on pure cultures. This possibility has been indicated already at the time of Pasteur and Tsenkovskiy, and great practical benefits were derived therefrom.

To assure the required direction of the work on species modifiability of microorganisms, the idea of generation (generatsiya), properly speaking, must be defined more precisely. Many microbiologists do not distinguish between the concepts of generation proper and generation (pokoleniye).

In the light of the Michurin-Lysenko theory, the change of generations of single-cell organisms in vegetative division must not be considered biologically equivalent to a change in the generation proper. The vegetative division of microorganisms can well be considered as a process of reproduction of like organisms only, a process analogous to the vegetative proliferation of cells of any tissue, of any single organ of a multicellular organism, i.e., as vegetative growth. We arrive at this conclusion also by the fact that many microorganisms in vegetative multiplication form chains or branching forms not divided into members. In general, the widespread idea must be re-examined that microbes, including bacteria, exist only in a state of isolation from each other, as independently living unicellular organisms. On solid substrates, both in nature and in artificial cultures, microbes live in multicellular colonies of many forms and structures. Even in liquid media, they are by far not always diffused throughout the medium in the form of isolated individuals, but form films, colonies on the walls, and precipitates which are often very characteristic.

In sporiforous microbes, the period of development of microbe cells from spore to spore can still be treated as a generation proper, the more so since in microbe cells, the process of spore formation occurs, as a rule, only in

every few generations, [arising] as a result of vegetative division. As far as nonsporiferous bacteria are concerned, very few data has been collected so far on the question of which forms in their ontogenetic development can be considered reproductive (similar to spores). Here we must recall the so-called gonidia and arthrospores described by various authors who had studied the forms of bacteria in aging cultures. Without further precision of the concept of generation proper, we cannot clarify the concept of ontogenetic development of bacteria. Naturally, this question, as all other questions of species modifiability, must be treated only in the process of practical solution of important tasks, otherwise, the matter will degenerate into academic speculation.

The collected material on the precell forms of microbes acquires special interest in connection with the notable work of Professor O. B. Lepeshinskaya on the generation of cells from living matter devoid of cell structure. T. D. Lysenko characterizes the importance of Lepeshinskaya's work for the correct understanding of development of the organic world and the transformation of species (philogenesis) as follows:

"Can one imagine that a cell of the wheat plant will transform itself into a cell of the rye plant? I cannot imagine it. This cannot be. We imagine it as follows: In the body of the organism of the wheat plant, under the influence of appropriate conditions of life, granules of rye are originated. But this origination does not occur through the transformation of the old into the new; in this case, of a wheat cell into a rye cell, but through the generation of a granule of the new organism in the depth of the given organism from a substance which has no cell structure. These granules at first also have no cell structure but then form cells and rudiments of the new organism. This is how Lepeshinskaya explains the theory of species formation."

It is no less difficult to imagine that even a single-cell organism of one species should, in the process of the usual vegetative propagation (division) be transformed into a single-cell organism of another species.

Many Soviet and foreign researchers (V. V. Suknev, N. A. Krasil'nikov, M. D. Utenkov, G. P. Kalina, V. D. Timakov, N. N. Zhukov-Verezhnikov, M. M. Pokrovskaya, V. A. Krestovinkova, and others) have indisputably demonstrated the following in experiments with various species of microorganisms.

The precell particles of microbe cells are capable of maintaining themselves even outside the cells and under favorable conditions, develop into a complete microbe cell if they get into the external medium due to disintegration of the cells. In the process of development and regeneration of precell particles, dependent on the conditions of life, cells are formed which are close to the original culture, but frequently, even as a rule, cells are formed which differ to some degree from the original microbe.

In this manner, direct experiments on microbiological objects have shown that the generation of elements with new qualities in the depths of the old species starts already at a very early stage of cell development, in the process of the development of the precell forms into single-cell organisms, i.e., into microbe cells. The disintegration of microbe cells under the influence of unfavorable conditions takes place not only in artificial laboratory surroundings but literally anywhere in an external medium, in the soil, in water, and in the organism of plants and animals. If we consider this, it must be realized that the process of development of precell forms of microbes under conditions which are not favorable to the nature of the old species, i.e., to its heredity, is the main source of the formation of new stocks and species in the world of microorganisms.

The facts and observations on the origin and development of precell filterable forms of microbes afford wide possibilities for experimental study on a new object, i.e., microorganisms, of the process of species rebuilding itself, i.e., the process of origination in the depths of the old species of elements or rudiments with features and properties of a new species. A detailed exposition of data on filterable forms of microbes and an analysis of these data have been supplied by G. P. Kalina (4) and by the author of this article. I shall limit myself to a short listing of the methods of regenerating precell forms:

The following methods are suggested for the development of precell filterable forms into microbe cells:

1. Growing in the presence of sarcinae or staphylococci (so-called feeder-strain method of Sukmev). Among the products of life activity of these microbes, there are apparently substances which are essential for the development of filterable forms.

2. Instead of live microbe-feeder strains, an emulsion of dead microbes of some species may be used. The possibility of using this method has been indicated in the reports of Matveyevskiy and Kalina.

3. Prolonged growing (for several weeks) at different temperatures, on different nutrient media.

4. Successive infection in series of animals with material containing filterable forms, or successive reseeding on artificial media.

In the work on the study of heredity and its modifiability, one must also always take into consideration that individual microbe cells, like individual specimens of higher plants and animals, do not change with uniform speed or to a uniform extent, and that in different specimens, new features and properties are retained by the offspring to a different degree. Selection or, in the language of the microbiologist, breeding, is an essential element of the work, not only for the establishment of new features and properties in individuals belonging to certain common groups, but also for strengthening and hereditary fixation of the newly acquired characteristics.

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